A PEPTIDE FRACTION IN LIVER*

By HENRY BORSOOK, CLARA L. DEASY, A. J. HAAGEN-SMIT, GEOFFREY KEIGHLEY, AND PETER H. LOWY

(From the Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena)

(Received for publication, December 29, 1948)

We reported in a preliminary communication (1) the isolation of a peptide fraction from guinea pig liver. The following points of interest appeared at once: many different amino acids were obtained on hydrolysis; the peptide fraction contained most of the indispensable amino acids, which indicated that it probably is important in protein metabolism; when guinea pig liver homogenate was incubated with C^{14}-labeled glycine, leucine, or lysine, these were rapidly incorporated into this peptide fraction,[1] which is further evidence that it is metabolically active; the peptide fraction had not been described hitherto; a fraction containing one or more large peptides can be separated from so complex a mixture as liver homogenate by starch chromatography.

We have not yet established whether the peptide fraction obtained by chromatographic isolation is a mixture which chromatographs as a unit or whether it consists of a single peptide. The following evidence favors the latter alternative. The fraction was precipitated by picric, flavianic, or trichloroacetic acids; after removal of the precipitating acid the fraction behaved the same chromatographically as it did before. Its amino acid composition was not demonstrably different before or after precipitation with picric acid nor before or after precipitation with ether from aqueous ethanol. The same chromatographic peptide fraction was found in the liver of fish, beef, guinea pig, hog, horse, lamb, and rat, and in guinea pig blood, diaphragm, heart, kidney, and spleen; this fraction from such widely different sources contained the same amino acids and their relative proportions were similar. Furthermore we have found this fraction to be one of the major products arising in the peptic hydrolysis of bovine serum albumin, bovine γ-globulin, casein, fibrin, insulin, and ovalbumin. (The details of this finding will be presented in a later communication.) Data are presented below on the peptide fraction isolated from Witte's peptone

* This work is a part of that done under contract with and joint sponsorship of the Office of Naval Research, United States Navy Department, and the United States Atomic Energy Commission. The C^{14} used in this investigation to label amino acids was supplied by the Monsanto Chemical Company, Clinton Laboratories, Oak Ridge, Tennessee, and obtained on allocation from the United States Atomic Energy Commission.

[1] The details of these findings will be reported later.

(which is derived from a peptic hydrolysate of fibrin). Its chemical behavior and amino acid composition were similar to those of the same chromatographic fraction obtained from liver. The foregoing evidence indicates that the chromatographic fraction is an operational entity; tentatively it will be considered as such pending further evidence from chemical and metabolic studies now in hand.

We have designated this fraction as Peptide A. There are other metabolically active peptides in liver which we are now engaged in identifying.

In view of the fact that this peptide fraction is an operational entity in a number of respects, the present communication describes methods of isolation, some of its physical and chemical properties, the amount in the liver of a number of different animals and in some guinea pig tissues, and its amino acid composition as obtained by different methods and from different sources.

Procedure

The tissues used were those of adult guinea pigs and of white rats procured from several commercial and laboratory sources, of fish from a cannery,[2] and of abattoir beef, hog, horse, and lamb.

The guinea pigs and rats were killed by stunning and bled. The guinea pig tissues and rat livers were chilled and worked up immediately. The livers of other animals were frozen with solid CO_2 a few minutes after removal from the animals and kept frozen until they were worked up.

The tissue was first disintegrated in a Waring blendor and then homogenized in the apparatus of Potter and Elvehjem (2). The homogenate was diluted with 25 times its weight of water, the pH adjusted to 5.0, and then boiled for 10 minutes. The coagulated protein was removed by filtration and reextracted three times by boiling with water. The original filtrate and the washings were combined and concentrated to dryness by distillation *in vacuo* at a bath temperature of 50°.

The residue was then chromatographed on starch by the method of Stein and Moore (3). Amino acids, peptides, and other substances are eluted from a starch column by a continuous flow of a selected solvent. Different substances appear in the eluent, separately or in groups in a definite order. The eluent is collected in fractions. When these are analyzed with ninhydrin reagent, the intensity of color is proportional to the amount of amino nitrogen in the fractions. This intensity of color (conveniently expressed in terms of amino nitrogen on the basis of an amino acid standard), when plotted consecutively against volume of effluent which has emerged, gives

[2] For which we wish to thank Dr. E. Geiger, Van Camp Laboratories, Terminal Island, California.

a "spectrum" consisting of bands of varying width and height (Fig. 4). The total color in the band tells the amount of substance.

Chromatography of Tissue Extracts: Peptide A Fraction—The following is an example of the quantities of tissue, starch,[3] and solvents used. The dried residue of the non-protein filtrate from 25 gm. of liver was dissolved in 1.5 ml. of N HCl and 30 ml. of a solvent mixture consisting of 1 part of 0.1 N HCl, 2 parts of *n*-propanol, and 1 part of *n*-butanol (4). This solution was transferred to the top of a column containing 1 kilo of starch, and forced into it with slight air pressure; the sides of the column were washed down with three small portions of the HCl-propanol-butanol mixture, each washing being forced into the top of the column before the next was added. The same solvent mixture was used for elution.

The dimensions of the starch column were 80 × 300 mm. The rate of flow of eluent was 75 to 90 ml. per hour. This rate was obtained by adjusting the pressure to 10 to 15 cm. of Hg, applied by either air or nitrogen.

0.5 ml. aliquots of the fractions were analyzed by the modification by Moore and Stein (5) of the ninhydrin method.

Chromatography of Hydrolysates—For the determination of the amino acid composition of the Peptide A fraction, 2 to 8 mg. were hydrolyzed by refluxing with 20 per cent HCl at a bath temperature of 150° for 20 hours. After removal of the excess acid the dry residue was chromatographed on a 7 to 10 × 300 mm. starch column, containing 21 to 25 gm. of starch, with the HCl-propanol-butanol solvent mixture as eluent. The rate of flow was adjusted to 1 to 2.5 ml. per hour; three fractions were collected per hour. After about 200 such fractions the following amino acids (if present) have been eluted, and in this order: leucine, isoleucine, phenylalanine, tryptophan, methionine, tyrosine, valine, proline, glutamic acid, alanine, threonine, aspartic acid, serine, and glycine.

At this point, it is possible by changing the eluent to a mixture consisting of 2 parts of *n*-propanol and 1 part of 0.5 N HCl to accelerate the emergence of ammonia, the bases, and cystine (4). In our experience the subsequent chromatogram was often ragged. This procedure was useful nevertheless; it indicated the presence of the bases and it permitted computation of the total amino nitrogen (reacting with ninhydrin) after acid hydrolysis. Other methods were used to establish the identity of the ammonia and amino acids emerging after the change in eluting solvent.

Chromatographic Isolation of Peptide A Fraction—The Peptide A fraction is eluted from the starch column by the HCl-propanol-butanol solvent mixture a short time before the first free amino acid, which is leucine (Fig.

[3] Two batches of white potato starch were used: one was obtained from the Amend Drug and Chemical Company, Inc., New York; the other was manufactured by the Idaho Potato Starch Company, Blackfoot, Idaho.

1). When the peptide fraction is rechromatographed, it emerges again in the same place (Fig. 2).

Distribution of Peptide A Fraction—Table I gives figures on the amounts of Peptide A fraction found in the livers of a number of animals and in a number of guinea pig tissues. The non-protein extracts were prepared and chromatographed as described above. The figures in the second

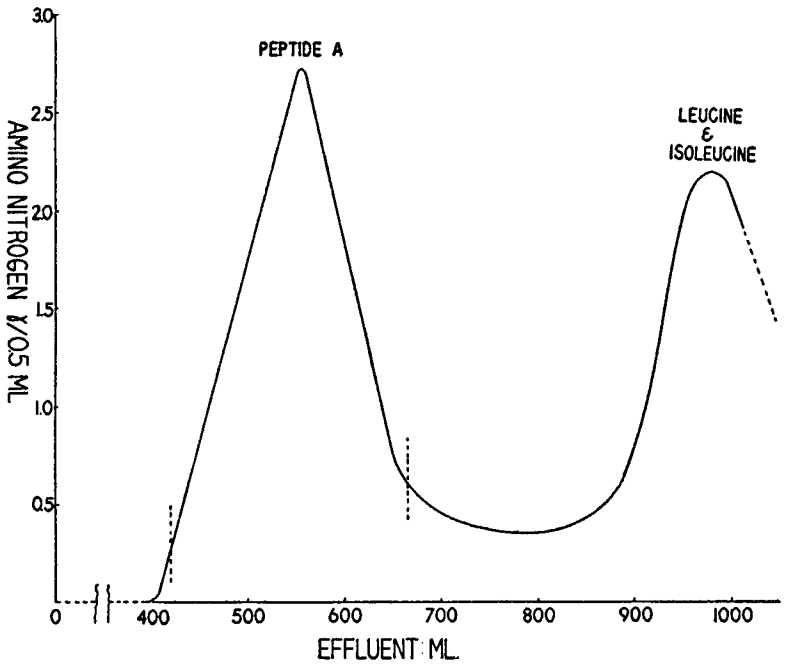

FIG. 1. Chromatographic separation of the Peptide A fraction from the non-protein filtrate of guinea pig liver. The first portion of the chromatogram of the non-protein filtrate from 23 gm. of guinea pig liver. Solvent, 1:2:1, 0.1 N HCl-*n*-propanol-*n*-butanol; column, 1000 gm. of starch, diameter 80 mm., height 300 mm. The eluent was collected in 32 ml. fractions; 0.5 ml. aliquots were analyzed. The concentration of amino nitrogen calculated from the ninhydrin color referred to that given by the leucine standards. One-half of the combined fractions between the vertical dotted lines was rechromatographed (see Fig. 2).

column were obtained by converting the total color given with the ninhydrin reagent in the fractions comprising the Peptide A band into amino nitrogen on the basis of the leucine standards. The figures in the last column were obtained from those in the second by applying the factor 6.5 γ of amino nitrogen = 1 mg. of peptide. This factor was given by preparations of the peptide fraction isolated by a number of different pro-

cedures described in the next section. The figures for mg. per cent of the peptide fraction are maximum values and probably are somewhat too high; a small admixture of material of low molecular weight giving color with the ninhydrin reagent would result in a great magnification of the amount of peptide estimated by applying the factor 6.5 γ = 1 mg. of peptide. This reservation does not, in view of all the evidence, invalidate the use of the ninhydrin values as an indication of the relative amounts of the

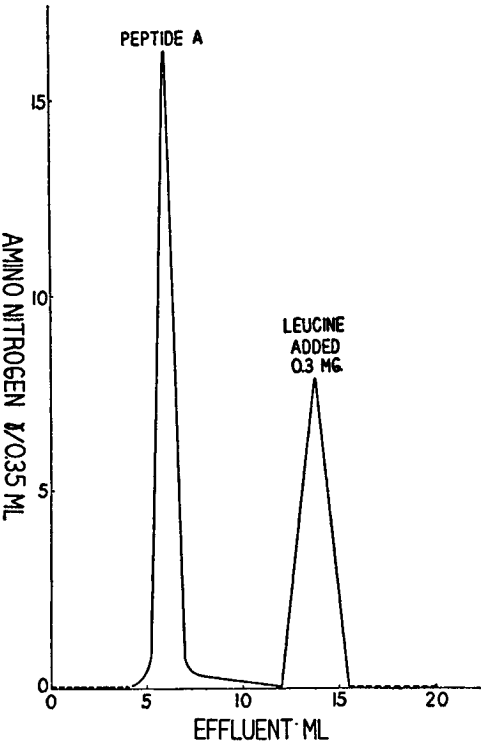

FIG. 2. The Peptide A fraction isolated as shown in Fig. 1 rechromatographed with 0.3 mg. of leucine added. Solvent, 1:2:1, 0.1 N HCl-*n*-propanol-*n*-butanol; column 21 gm. of starch, diameter 7 mm., height 275 mm. The eluent was collected in 0.35 ml. fractions; a whole fraction was analyzed.

peptide fraction in different tissues. On this basis the data in Table I indicate that liver contains large amounts of the Peptide A fraction; spleen is almost as rich, and there is little or none in striated skeletal muscle.

Physical and Chemical Properties and Chemical Isolation of Peptide A Fraction—The properties described in this section are those of the Peptide A fraction from guinea pig liver, rat liver, and from Witte's peptone. We found no difference between them. We shall in this section for convenience

refer to this fraction as if it were a single peptide; as stated above it may consist of a number of peptides of similar composition and behavior. The peptide is soluble in water; its hydrochloride is soluble in 80 per cent ethanol and in absolute methanol; it is precipitated from solution in the two latter solvents by ether. As was to be expected from its amino acid composition, it gives a picrate and a flavianate. It is precipitated by high concentrations (10 to 20 per cent) of trichloroacetic acid; dilute solutions of the peptide are less completely precipitated than concentrated solutions. It gives a purple biuret test, and positive tests with Millon's and Hopkins-Cole reagents.

TABLE I

Peptide A Fraction in Some Animal Tissues

Sources	Amino nitrogen, γ per 100 gm. tissue, wet weight	Mg. per 100 gm. tissue, wet weight*
Albacore liver	2600	400
Beef liver	3600	550
Guinea pig liver	3500	530
Hog liver	3350	510
Horse "	1200	180
Lamb "	1170	180
Rat "	2700	410
Guinea pig blood	115	18
" " diaphragm	Trace	Trace
" " heart	470	72
" " kidney	250	38
" " spleen	2100	320
" " striated muscle of abdominal wall	0	0

* The values in this column were obtained by applying the factor 6.5 γ of amino nitrogen (ninhydrin) = 1 mg. of Peptide A.

The foregoing physical and chemical properties provided several methods for isolating the peptide, either from starch and other materials in the HCl-propanol-butanol mixture in which it was eluted from the starch column, or, when Witte's peptone was the source, from other materials in the peptone mixture. The preparations obtained by the following isolation procedures are not to be construed as pure.

The chromatographic Peptide A fraction of 25 gm. of either guinea pig or rat liver was dried in a current of air. The residue was stirred in 10 ml. of 95 per cent ethanol, and water was then added dropwise until practically all the material was dissolved. This was centrifuged and to the clear supernatant solution ether was added dropwise until a heavy precipitate

appeared. The precipitate was submitted to the same procedure twice more, then washed with ether, and dried. The dried material, assayed with the ninhydrin reagent, gave 6.5 γ of amino nitrogen per mg. It gave before hydrolysis the same chromatogram as the original Peptide A fraction. Fig. 5 is the chromatogram of the preparation from rat liver after it was hydrolyzed.

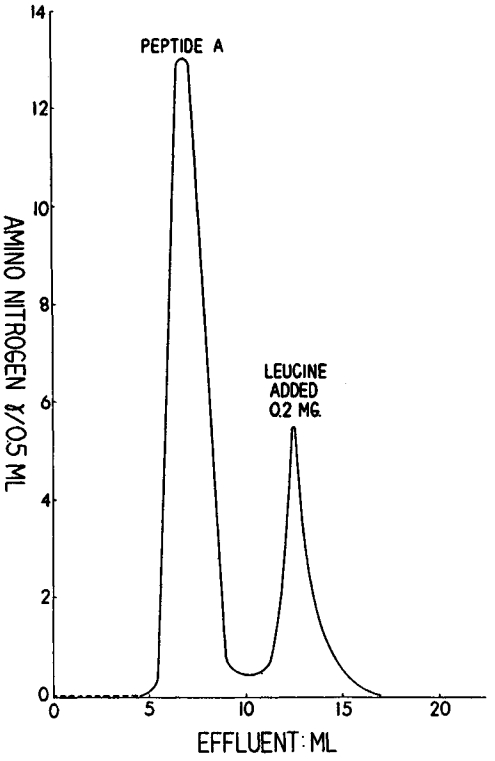

FIG. 3. Chromatogram of the Peptide A fraction isolated via the picrate from Witte's peptone as described in the text. 8 mg. chromatographed with 0.2 mg. of added leucine. Solvent, 1:2:1, 0.1 N HCl-*n*-propanol-*n*-butanol; column, 25 gm. of starch, diameter 9 mm., height 290 mm. The eluent was collected in 0.6 ml. fractions; 0.5 ml. aliquots were analyzed.

Peptide A was isolated as the picrate from the starch column eluate of the non-protein fraction of guinea pig liver and from Witte's peptone which had not been chromatographed. After the eluent fraction was evaporated to dryness, the subsequent procedure was the same in the isolation from both sources. To the aqueous solution of the eluent fraction or of Witte's peptone a saturated aqueous solution of picric acid was added until maxi-

TABLE II

Comparison of Amino Acid Composition of Peptide A Fraction from Different Sources

Source	Treatment of Peptide A before hydrolysis	Amino nitrogen in aliquot				Ratio of amino nitrogen in other bands to that in leucine + isoleucine bands				
		Before hydrolysis	After hydrolysis	Ratio, after hydrolysis to before hydrolysis	In leucine + isoleucine bands*	Methionine + tyrosine†	Glutamic acid + alanine	Threonine + aspartic acid	Serine	Glycine
		γ	γ		γ					
Albacore liver	None				64.4	0.60	1.38	1.18		
Beef liver	"	30.2	403	13.3	53.7	0.76	1.39	1.08	0.41	0.59
Guinea pig liver	"	95.9	1288	13.4	173.8	0.86	1.48	1.19	0.41	0.64
" " "	"				59.5	0.64	1.29	0.92	0.31	0.59
" " "	"	30.1	413	13.7	54.9	0.60	1.37	1.16	0.44	0.78
" " "	Isolated as picrate‡	22.9	363	15.8	50.4	0.62	1.18	1.03	0.44	0.79
Hog liver	None	28.4	454	15.9	64.2	0.65	1.36	1.02	0.41	0.61
Horse liver	"	43.2	655	15.1	97.3	0.57	1.32	1.05	0.42	0.67
Lamb "	"	38.9	552	14.1	76.4	0.83	1.24	1.02	0.36	0.71
Rat "	"	38.6	529	14.7	67.9	0.60	1.43	0.94	0.41	0.73
" "	"	32.1	461	14.3	67.5	0.74	1.35	0.98	0.38	0.67
" "	Isolated from solution in ethanol§	13.0	191	14.6	25.4	0.79	1.31	1.08	0.44	0.76
" "	Same	13.0	206	15.8	24.8	0.62	1.41	1.18	0.43	0.78
Witte's peptone	Isolated as picrate‖	13.0	179	13.7	23.1	0.73	1.52	1.48	0.43	0.78
Guinea pig blood	None				79.4	0.75		1.07	0.37	0.66
" " "	"				18.2	0.58	1.29		0.36	0.58
" " kidney	"				52.5	0.52	1.38	1.08	0.47	0.68
" " spleen	"				44.9	0.52	1.25	1.01		

* Phenylalanine was omitted from the designation of the leucine-isoleucine band because only questionable amounts were found by colorimetric, ultraviolet, and chromatographic methods.

† The valine was omitted from the designation of the methionine-tyrosine band because the values found by colorimetric determination of the methionine and colorimetric and ultraviolet determination of the tyrosine accounted for practically all the amino nitrogen in the band.

‡ Prepared as follows: the Peptide A fraction precipitated with picric acid, the picric acid removed from the precipitate with ether, and the residue hydrolyzed.

§ Prepared as follows: the Peptide A fraction evaporated to dryness; residue dissolved in 80 per cent ethanol from which it was precipitated with ether. Solution in 80 per cent ethanol and precipitation with ether repeated twice more, the residue dried, and 2 mg. hydrolyzed and the hydrolysate chromatographed. The amino nitrogen value before hydrolysis was obtained by direct assay of the isolated preparation.

‖ Aqueous solution of Witte's peptone precipitated with picric acid; the picric acid removed from the precipitate with ether; the residue chromatographed; the Peptide A fraction evaporated to dryness in air and dissolved in 80 per cent ethanol, from which it was precipitated with ether; and 2 mg. of the dried precipitate hydrolyzed and chromatographed. The amino nitrogen value before hydrolysis was obtained by direct assay on the isolated preparation.

mum precipitation had occurred. After standing overnight in the refrigerator the suspension was centrifuged, the precipitate dissolved in a minimum amount of HCl and the picric acid extracted with ether. Ethanol was then added to a final concentration of 80 per cent and the peptide precipitated with ether as described above. Data on these preparations are given in Figs. 3 and 6 and in Table II.

The simplest isolation from Witte's peptone was as follows. 200 mg., dissolved in 50 ml. of water, were precipitated with 10 per cent (final concentration) of trichloroacetic acid. The trichloroacetic acid was removed from the precipitate with ether, and the residue was dried with absolute ethanol and ether. The yield was 14 mg. The chromatogram before hydrolysis was the same as that of all other preparations of the Peptide A fraction; 6.5 γ of amino nitrogen (by ninhydrin) per mg. were obtained.

A preparation isolated from Witte's peptone via the flavianate gave the same amino nitrogen value and the same chromatogram. 2.0 gm. of Witte's peptone were suspended in 70 ml. of absolute methanol; 0.7 ml. of concentrated HCl was added and after thorough shaking the mixture was centrifuged. The precipitate was suspended in a fresh 70 ml. portion of methanol and 0.7 ml. of HCl and the extraction was repeated. In all, five such extractions were made, the residue from one extraction being used for the next. Twice the volume of absolute ether was added to the combined supernatant solutions. The precipitate was separated by centrifuging and then dried. It weighed 620 mg. 200 mg. of the dried precipitate were dissolved in 2 ml. of water, and to the solution were added 100 mg. of flavianic acid in 2 ml. of water. The gummy, orange precipitate was separated from the yellow supernatant liquid by centrifuging. 0.2 N H_2SO_4 was added to the precipitate until it was strongly acid and the flavianic acid was extracted with amyl alcohol. The clear residual solution was treated with $Ba(OH)_2$ to remove the sulfate and with CO_2 to remove the excess barium and was then evaporated to dryness. The yield was 30 mg.

Amino Acid Composition of Peptide A Fraction—The amino acid composition of the Peptide A fraction was determined by starch chromatography after acid hydrolysis as described above. The results are given in Figs. 4, 5, and 6 and in Table II. The material hydrolyzed was either that in the central region of the Peptide A band of the non-protein filtrate of a tissue or the preparations isolated as described in the previous section. Moore and Stein (5) give the positions of the different amino acids in the chromatogram obtained by elution first with HCl-propanol-butanol and then with propanol-HCl. We have used a number of tests to check these positions and have confirmed Moore and Stein completely. C^{14}-labeled leucine and glycine (and lysine, not shown in Figs. 4, 5, and 6) were added

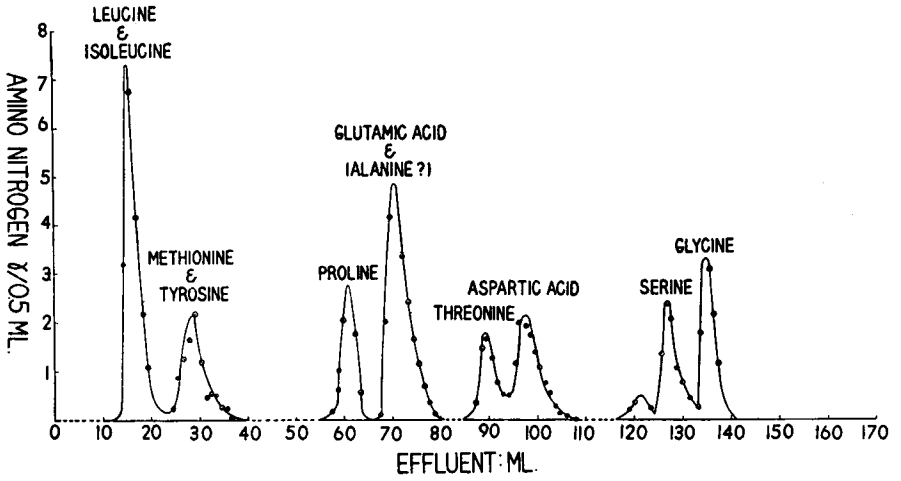

FIG. 4. Chromatogram of the hydrolysate of 20 per cent of the Peptide A fraction of guinea pig liver shown in Fig. 1. Solvent, 1:2:1, 0.1 N HCl-*n*-propanol-*n*-butanol; column, 25 gm. of starch, diameter 9 mm., height 298 mm. The eluent was collected in 0.55 ml. fractions; 0.5 ml. aliquots were analyzed. The portion of the chromatogram after the glycine band is not shown.

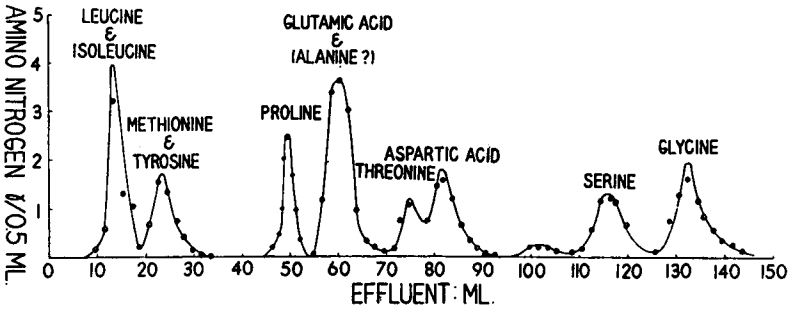

FIG. 5. Chromatogram of the hydrolyzed Peptide A fraction from rat liver first isolated from the chromatographic Peptide A fraction by dissolving the dried residue of the fraction in ethanol and then precipitating with ether as described in the text. 2 mg. of the dried precipitate were hydrolyzed and chromatographed. Solvent, 1:2:1, 0.1 N HCl-*n*-propanol-*n*-butanol; column, 25 gm. of starch, diameter 9 mm., height 279 mm. The eluent was collected in 0.9 ml. fractions; 0.5 ml. aliquots were analyzed. The portion of the chromatogram after the glycine band is not shown.

to aliquots of a hydrolysate and the coinciding radioactivity and ninhydrin peaks found in the fractions of eluent. Leucine, tyrosine, proline, threonine, aspartic acid, serine, and glycine were located by a loading procedure. A relatively large amount of one amino acid was added to an

aliquot of a hydrolysate; this loaded aliquot and another to which no addition was made were chromatographed at the same time on two separate columns. The chromatograms of the two portions of the hydrolysate were the same except for the heightened peak in one, corresponding to the amino acid added.

Proline gives a yellow color with the ninhydrin reagent. The presence in the peptide fraction (but not the location in the hydrolysis chromatogram) of tryptophan (6), methionine (7), and tyrosine (8), and arginine and histidine (9) was determined colorimetrically. The ultraviolet spectrum of the unhydrolyzed Peptide A fraction was measured and was practically completely accounted for by the content of tryptophan and tyrosine as found colorimetrically.

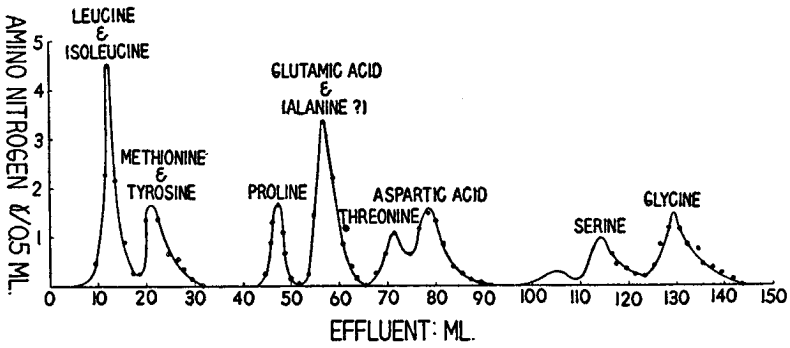

FIG. 6. Chromatogram of the Peptide A fraction from Witte's peptone, isolated via the picrate as described in the text. 2 mg. were hydrolyzed and then chromatographed. Solvent, 1:2:1, 0.1 N HCl-*n*-propanol-*n*-butanol; column, 25 gm. of starch, diameter 8 mm., height 295 mm. The eluent was collected in 0.9 ml. fractions; 0.5 ml. aliquots were analyzed. The portion of the chromatogram after the glycine band is no shown.

The presence of lysine was established also by the use of *lysineless Neurospora*, mutant 4545 (10).[4] Unhydrolyzed Peptide A fraction will not support growth of this mutant unless a small amount of free lysine is added to the culture medium. Hydrolyzed Peptide A fraction requires no such seeding. Evidently the minimum growth afforded by a trace of free lysine provides hydrolytic enzymes which liberate lysine from the peptide (or peptides) for further growth.

The presence of isoleucine in the leucine band was established by chromatography on starch according to Stein and Moore (3), with 1:1:0.284 *n*-butanol-benzyl alcohol-water as the eluting solvent. This solvent mix-

[4] We wish to thank Mr. E. Windsor of this laboratory for carrying out the lysine assays with the *Neurospora* mutant.

ture separates phenylalanine, leucine, and isoleucine. The presence of leucine was established by using C^{14}-labeled leucine as a tracer, and that of isoleucine by the loading procedure described above.

Filter paper chromatography with phenol and *s*-collidine (11) of the glutamic acid + alanine fractions gave several spots; among them were those which could be assigned to glutamic acid and alanine, but the picture was complicated by the presence of spots given by substances which were not identified.

The presence in the Peptide A fraction from every source listed in Table II of the following amino acids has been established by the methods referred to above: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tryptophan, and tyrosine. Chromatography on starch by elution with the *n*-butanol-benzyl alcohol-water solvent, which separates phenylalanine, leucine, and isoleucine (3), indicated that phenylalanine or some other amino acid which emerges before leucine may be present. If phenylalanine is present there is very little. To establish this point it will be necessary to prepare and highly purify much larger amounts of the peptide or peptides in the fraction than we have at present. The same considerations apply to cystine, valine, and other amino acids which may be present, as for example the substance giving the low rounded peak between aspartic acid and serine shown in Figures 4, 5, and 6.

Figs. 4, 5, and 6 are chromatograms of hydrolyzed Peptide A fraction from three different sources, guinea pig liver, rat liver, and Witte's peptone. They are clearly the same qualitatively. Similar chromatograms were given by hydrolyzed Peptide A from all the sources listed in Table I. These chromatograms indicated the same qualitative amino acid composition of the Peptide A fractions from different sources, isolated by different methods.

Table II summarizes data which indicate that the quantitative amino acid composition of Peptide A from different sources, isolated by different methods, was similar. Table II gives values of amino nitrogen before and after acid hydrolysis. Except those indicated in the foot-notes the amino nitrogen values before hydrolysis were obtained from the chromatogram of the Peptide A fraction. All the values after hydrolysis were obtained by adding the ninhydrin values of all the bands obtained by elution first with the HCl-propanol-butanol solvent followed by the propanol-HCl mixture. All the values of the ratio after hydrolysis to before hydrolysis fall within the range 13.3 to 15.9; the average is 14.5.

The concordance of the values of this ratio further supports treatment of the chromatographic Peptide A fraction as an operational entity. The value of 14.5 of the ratio of free amino nitrogen after hydrolysis to before

hydrolysis is within the range found for proteins. Thus Henriques and Gjaldbaek (12) by formol titration found values for dried egg white, casein, edestine, gelatin, and Witte's peptone of 14.6, 8.8, 24.4, 22.7, and 5.6 respectively. We may conclude, therefore, that most of the Peptide A fraction chromogenic with the ninhydrin reagent consists of polypeptide material of large molecular weight.

Table II gives the ratios of the amino nitrogen in the main bands to that of leucine + isoleucine. The figures in Table II show that the Peptide A fractions of different origin appear to have similar amino acid composition. This statement applies not only to the comparisons in Table II but also to their content of lysine. The *Neurospora* assays indicated that they all contain 9 to 10 per cent of lysine. This value probably is low, as arginine and glutamic acid, both of which are present in the peptide, inhibit this mutant. In every case it was necessary to add a trace of free lysine to initiate growth, and there was the same lag of 24 to 48 hours before rapid growth began.

Ratios of the amino acid groups were compared in Table II because we do not consider the Peptide A fractions to have been pure enough to warrant comparison of the content of individual amino acids expressed as absolute amounts. It is premature, we feel, to stress the differences in the ratio of the same amino acid group. Stein and Moore (3) in replicate analyses of purified proteins show divergencies of 10 per cent between extreme values for one amino acid. Each analysis in Table II was done on a specimen of Peptide A fraction obtained from a different animal. The notable finding is that the fraction obtained from widely different sources was so similar with respect to all five amino acid groups compared to leucine plus isoleucine and with respect to the ratio of amino nitrogen before and after hydrolysis. *A priori* no such similarity was to be expected.

DISCUSSION

The following considerations bear on the questions of the purity of the Peptide A fraction from any one source and the identity or difference of this fraction obtained from different sources. Contamination by free amino acids is excluded because these emerge from the starch column (with the eluting solvent employed) after the Peptide A fraction. The Peptide A fraction is precipitated by 10 per cent trichloroacetic acid; small peptides are thereby excluded as contaminants. Further evidence in the same direction is that a number of dipeptides and glutathione emerge after leucine, whereas the Peptide A fraction emerges before leucine. The Peptide A fraction is precipitated by picric and flavianic acids; contamination by peptides not containing basic amino acids is thereby excluded.

The amino acid composition of the original chromatographic fraction

was not demonstrably different from that of the fraction after precipitation either with picric acid or with ether from aqueous ethanol. Also the ratio of free amino nitrogen (with ninhydrin reagent) before and after hydrolysis was the same for the three materials. This ratio is of the order to be expected of a peptide containing the fifteen (or more) amino acids present in the fraction. Therefore, if the Peptide A fraction is a mixture, it is a mixture of similar large peptides.

SUMMARY

1. The chromatographic isolation of a peptide fraction in liver, designated Peptide A, is described. Several chemical methods of isolating it are described. This fraction contains one or more large peptides.

2. The presence of the following amino acids in the Peptide A fraction has been established: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tryptophan, and tyrosine. Additional amino acids may be present.

3. The Peptide A fraction has been isolated from the livers of albacore, beef, guinea pig, hog, horse, lamb, and rat, from guinea pig blood, heart, kidney, and spleen, and from Witte's peptone. Liver and spleen are the richest sources; there is much less in blood, heart, and kidney and very little or none in diaphragm and striated muscle of the abdominal wall.

4. The Peptide A fraction isolated from different sources contains all of the above amino acids. Their proportions are not demonstrably different within the limits of precision of the analytical methods used.

BIBLIOGRAPHY

1. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, **174**, 1041 (1948).
2. Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, **114**, 495 (1936).
3. Stein, W. H., and Moore, S., *J. Biol. Chem.*, **176**, 337 (1948).
4. Moore, S., and Stein, W. H., *J. Biol. Chem.*, **178**, 53 (1949).
5. Moore, S., and Stein, W. H., *J. Biol. Chem.*, **176**, 367 (1948).
6. Carpenter, D. C., *Anal. Chem.*, **20**, 536 (1948).
7. Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, **169**, 247 (1947).
8. Bernhart, F. W., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.*, **123**, p. x (1938).
9. Macpherson, H. T., *Biochem. J.*, **36**, 59 (1942).
10. Doermann, A. H., *J. Biol. Chem.*, **160**, 95 (1945).
11. Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).
12. Henriques, V., and Gjaldbaek, K., *Z. physiol. Chem.*, **75**, 363 (1911).